REMOVAL OF RADIOSTRONTIUM FROM MILK WITH TRICALCIUM PHOSPHATE AND RESULTING CHANGES IN MILK COMPOSITION [1]

L. F. EDMONDSON, A. R. LANDGREBE, A. M. SADLER, AND H. E. WALTER

Dairy Products Laboratory, Eastern Utilization Research and Development Division, USDA
Washington, D.C.

ABSTRACT

The effectiveness of tricalcium phosphate in removing Sr-85 from milk and the effects of the procedure on milk composition were studied. The phosphate was added to milk at the rate of 80 g per liter and the resulting precipitate, containing Sr-85, was removed by centrifugation.

Heating milk to 70 C for 30 min, immediately following addition of phosphate, resulted in removal of 85% of the Sr-85.

When the pH of milk was adjusted to 5.4 with H_3PO_4, then to 8.2 with KOH before adding phosphate, 90% removal of Sr-85 was obtained by heating to 70 C for only 3 min. When phosphated milk (pH 6.6) was stirred at 5 C for 24 hr, only 60% of the Sr-85 was removed.

All procedures utilizing calcium phosphate precipitation reduced the milk nitrogen from 23 to 38%. Usually, more than 45% of the milk calcium, in addition to the added phosphate calcium, was precipitated. Also, a decrease in milk phosphorus occurred in some instances.

Several papers have been published concerning the use of ion exchange resins for removing radiostrontium from milk. It has been demonstrated by use of fixed bed ion exchange columns that 95% of strontium 90 can be removed on a pilot plant scale (1). Regeneration of the resins with a mixed salt solution having the proper composition results in insignificant changes in the milk composition. The flavor of the milk was shown to compare favorably with control samples.

A brief report has also been given by Silverman (2) on the use of calcium phosphate for co-precipitation of radiostrontium from milk. He added up to 80 g of calcium phosphate per liter of milk while heating to 60 C and above, for one-half hour. The milk was centrifuged at the end of the heating period to remove the added calcium phosphate and the radiostrontium. Up to 95% removal of strontium 85 was reported. The milk was labeled in vitro with the isotope. The form of calcium phosphate was not specified

Stirring the phosphate-milk mixture for one-half hour at 62 C, followed by centrifugation to remove the precipitate, is essentially a long-hold pasteurization procedure.

Research with the calcium phosphate precipitation procedure is the subject of this

paper. It was our purpose to investigate variables which might reduce the phosphate-milk contact heating time so that the processing could be done continuously and to study changes in milk composition as a result of the phosphate treatment.

MATERIALS AND METHODS

Preliminary work showed that only the tricalcium phosphate was suitable. The di- and mono- forms either resulted in acid precipitation of milk proteins or removed very little strontium. Consequently, the tri-basic form (technical grade from Baker and Adamson) was used for subsequent studies.

Raw whole milk labeled in vivo with strontium 85 was obtained from a Holstein cow given oral doses of 1 mc from 24 to 48 hr before collecting the milk. The activity in the milk ranged from 0.5 to 1.0 μc per liter. Milk labeled in vitro with the isotope was obtained by adding approximately 1 μc per liter and holding for 48 hr at 5 C. All data reported in this paper (except that listed in Table 1) were obtained by using in vivo labeled milk.

After establishing the quantity of phosphate needed for effective precipitation of strontium, and determining the effect of method of labeling milk with strontium 85 on removal by calcium phosphate, a series of experiments was carried out to study the effects of temperature, pH, and phosphate-milk contact time on the removal of radiostrontium. The phosphate was added to

TABLE 1

Effect of heating, and method of labeling milk with strontium 85 on its removal with calcium phosphate [a]

Method of la- beling	Tempera- ture	Holding time	% Sr-85 removed
	(C)	(min)	
In vivo	60	15	81.4
In vitro [b]	60	15	92.8
In vivo	80	15	87.6
In vitro [b]	80	15	95.2
In vivo	60	60	90.3
In vivo	80	60	94.4

[a] Eighty grams per liter of $Ca_3(PO_4)_2$ were added.
[b] Milk was equilibrated for 48 hr after adding Sr-85.

milk with thorough stirring during a 2-min interval after adjusting the milk to predetermined temperatures. The pH of milk was adjusted, when desired, by adding citric acid, phosphoric acid, or potassium hydroxide before heating.

Single runs were made for the experiments performed in this study, except in the series mentioned below.

Effect of pH of milk. Raw whole milk was divided into four one-liter lots, each of which was adjusted to one of the following pH levels: 5.4, 6.7 (normal milk), 7.6, and 8.2. After heating to 61 C (the lot at pH 5.4 was heated only to 52 C because of sensitivity to coagulation at elevated temperatures), 80 g of $Ca_3(PO_4)_2$ were added. Stirring continued for 1 hr during which time 50-ml samples were withdrawn after 7.5, 15, 30, and 60 min. These were cooled to room temperature and centrifuged for 5 min at 2,000 rpm in an International Model V centrifuge having a 20-in. maximum diameter head. Fifteen-milliliter samples, in duplicate, of the supernatant were assayed for strontium 85 with a single channel gamma scintillation analyzer.

Procedure when pH was readjusted before phosphate addition. Four one-liter portions of raw whole milk were used. Two of these were acidified to a pH of 5.4, one with 8.5% H_3PO_4 and the other with 0.75 M citric acid. These were then immediately readjusted to a pH of 8.2 with KOH. Of the remaining lots one was adjusted to pH 8.2 without prior acidification, and the other used as a control. After heating to 70 C, 80 g of phosphate were added to each lot. Samples were withdrawn after 2, 4, 6, 8, 10, 12, and 14 min, cooled, centrifuged, and counted as described above. Duplicate runs were made at each pH level, except for the sample acidified with citric acid. The averages are plotted in Figure 2 for each of the three
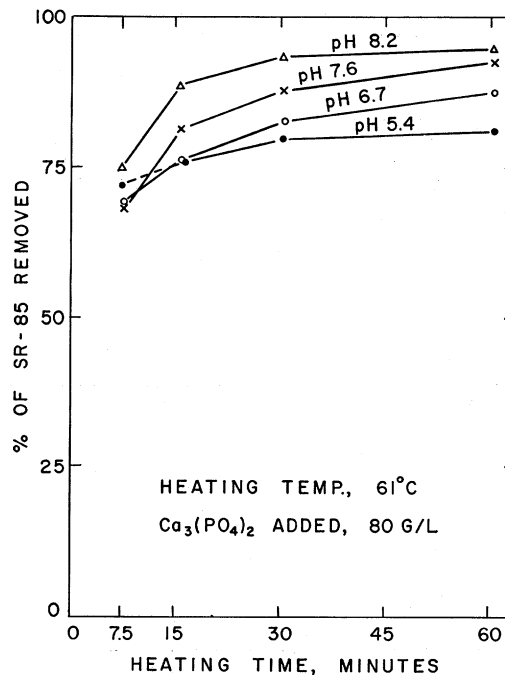

FIG. 1. Effect of pH and heat treatment on the co-precipitation of Sr-85 in milk with tricalcium phosphate.
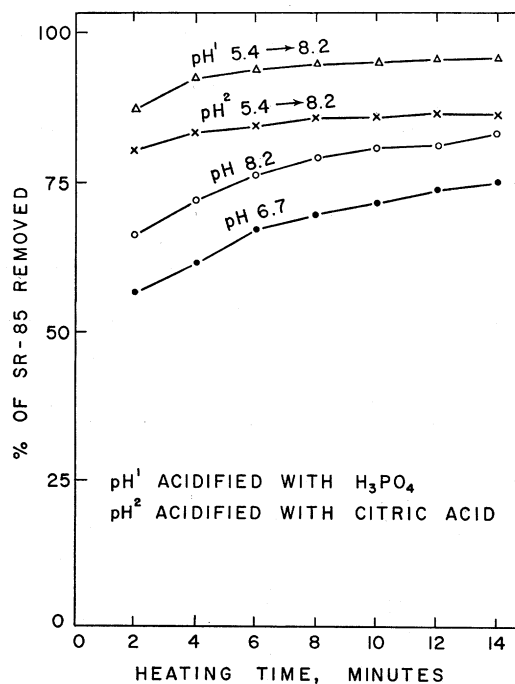

FIG. 2. Effect of pH adjustments on the co-precipitation of Sr-85 in milk with tricalcium phosphate. Heating temperature, 70 C.

other pH levels. The statistical analyses for 21 sets of duplicates (seven heating times and three pH levels) are given in the Discussion section.

Effect of phosphate addition on changes in milk composition. Four methods of treating milk with the calcium phosphate were employed to determine the effects on changes in the nitrogen, calcium, and phosphorus content: 1) Normal milk was heated to 70 C before adding the phosphate. Samples were withdrawn after 10 and 30 min, cooled, centrifuged to remove the phosphate, and analyzed for nitrogen, calcium, and phosphorus; 2) the pH of the milk was adjusted to 8.2 before adding the phosphate and heating to 70 C. Samples were taken after 2 and 10 min for analyses; 3) milk was first acidified to pH 5.4 with H_3PO_4, then readjusted to 8.2 with KOH before heating. Samples were taken after 2 and 10 min for analyses; 4) the phosphate was added to normal milk and stirred during refrigeration at 5 C for periods up to 19 hr before centrifuging and sampling.

Duplicate 50-ml samples in all of these runs were used for centrifuging. Analyses for each of the three elements were also done in duplicate. The results are reported as averages.

RESULTS AND DISCUSSION

Initial trials, carried out at 60 C for one-half hour, showed that it is necessary to add about 80 g of calcium phosphate per liter of milk to approach 90% removal of strontium. The use of 40 g per liter resulted in less than 70% removal. This difference is apparently due to a mass action effect.

Preliminary trials also included comparisons of the removal of strontium 85 from milks labeled in vivo and in vitro with this isotope. The results, given in Table 1, show that considerably less removal was obtained when milk was labeled in vivo than when labeled by direct addition of the isotope. This work was carried out by using milk at its normal pH. As a result of the preliminary information, further investigations were made by using milk labeled in vivo with strontium 85, and by adding 80 g of tricalcium phosphate per liter.

The effect of the pH of milk on the removal of strontium is shown in Figure 1. The amount and rate of strontium precipitation depends on two factors, both of which are pH-dependent: 1) the binding of strontium to casein, and 2) the solubility of calcium phosphate with which it co-precipitates. Since the degree of binding to casein is less at pH 5.4 than at higher pH levels, it is more readily available for co-precipitation. Initially (after 7.5 min. of heating), the effect

of pH on strontium precipitation was less than after extended heating times. The increase in removal observed with increasing pH, after prolonged heating, reflects the equilibrium solubility of strontium phosphate (in drawing conclusions from the data one must consider that the trials at pH 5.4 were carried out at 52 C, whereas all others were performed at 61 C). Heating for 30 min at pH 8.2 resulted in 94% removal; at pH 5.4 only 80% was removed. At elevated pH levels strontium is complexed with caseinate to a greater extent. The transition from this state to the co-precipitate is relatively slow, as indicated by the initial steepness of the curves at pH 6.7 and above, compared to that at pH 5.4.

Figure 2 shows the results of a series of runs made to determine the effect of acidification and readjustment of pH on the removal of strontium. Before discussing the results an indication of the error involved in making these runs will be given by considering statistical analysis of the data for which duplicate runs were made. The following tabulation, which lists the 95% confidence limits by pH treatments, shows that the precision is definitely a function of pH:

pH	$t_{.05}s$
	(%)
6.7	6.70
8.2	4.16
5.4 → 8.2 [a]	1.57

[a] Acidified with H_3PO_4 before readjustment of pH to 8.2.

The variances between the three pH treatments were significantly different. The 95% confidence limits listed in the next table show that the precision is also a function of heating time. These computations were made from the pooled values over all sets of duplicates.

Heating time	$t_{.05}s$	Heating time	$t_{.05}s$
(min)	*(%)*	*(min)*	*(%)*
2	9.23	10	5.63
4	7.42	12	4.95
6	5.63	14	4.09
8	5.29		

The amount of strontium removed (co-precipitated) was increased by first acidifying the milk to a pH of 5.4 before adjusting to pH 8.2. The use of phosphoric acid for acidifying resulted in greater precipitation than citric acid. Acidification results in a marked shift in strontium from the colloidal caseinate complex to an ionic or dissolved form. Readjustment of

the pH to 8.2 reverses this effect. However, rather than recombining, as in the initial state, strontium complexes or compounds are formed which are more readily precipitated after adding the calcium phosphate than when no prior acidification is used. When acidified with phosphoric acid, about 90% removal was obtained after heating for 3 min. At pH 8.2 without acidifying only 71% was removed. The enhanced removal with H_3PO_4, compared with citric acid, is probably due to the lesser solubility of calcium phosphates. Citrate tends to form soluble complexes with the alkaline earths.

The effect of calcium phosphate addition, in combination with heat treatments and pH adjustments, on changes in milk constituents is shown in Table 2. All treatments resulted in nitrogen losses ranging from 23 to 38%. The calcium losses ranged from 35 to 73% of the total milk calcium, except the last treatment (Lot 4), which resulted in a 15% increase. The treatments given Lots 1 and 2 resulted in phosphorus losses whereas Lots 3 and 4 showed gains. The calcium and phosphorus losses occurred even though a large quantity of calcium phosphate (80 g per liter) was added to the milk. This addition apparently causes a precipitation of a portion of the calcium caseinate–phosphate complex, the amount depending on the pH and heat treatment given the milk.

The increase in the phosphorus content of samples from Lot 3 was due to H_3PO_4 being used for acidifying the milk. The precipitating action of the phosphate was much less at low temperature (Lot 4); in fact, increases in calcium and phosphorus resulted from stirring for 19 hr at 5 C.

The sediments of all of the samples contained about 50% moisture and from 0.7 to 1.1% nitrogen. One liter of milk, containing 80 g of calcium phosphate, yielded about 160 g of moist sediment.

The pH of normal milk decreased from 0.2 to 0.4 unit during the heating periods employed in these experiments. The pH of milk adjusted to 8.2 decreased from 0.6 to 1.0 pH unit during the heating periods.

The procedure whereby milk was first acidified to pH 5.4 with H_3PO_4, followed by adjustment to pH 8.2 before adding the phosphate and heating, resulted in the greatest removal of strontium (Figure 2 and Table 2, Lot 3). This procedure might conceivably be developed into a continuous operation. The disadvantage, however, is the necessity to make three separate pH adjustments during and after processing for strontium removal. The removal obtained by mixing the phosphate with milk at low temperature (5 C) did not exceed 60%.

Flavor evaluation of some of the samples showed that they were not acceptable for beverage purposes. For this evaluation a calcium phosphate–milk mixture was stirred for one-half hour at 62 C, followed by centrifugation to remove the precipitate. The average flavor score given by a ten-man panel was 31.3 for the treated sample and 35.9 for the control. The panel was instructed to use a scale ranging from 31 to 40 points. Most of the panel members criticized the treated sample for an oxidized flavor. It is probable that the oxidation was the result of iron impurities in the added calcium phosphate.

From the data obtained in this work, several drawbacks to the precipitation procedure for removing radiostrontium from milk are apparent: 1) the loss in N, Ca, and P in the sediment after centrifuging; 2) poor flavor quality; and 3) the necessity to add a large quantity of calcium phosphate—almost 1 g per gram of

TABLE 2

Effect of adding calcium phosphate to milk on loss of N, Ca, and P in sediment after centrifuging

| Lot no. | pH adjustment | Milk treatment Heating | | % of Constituents lost in sediment | | | % Sr-85 removed |
		Time	Temp	N	Ca	P	
		(min)	*(C)*				
1	None	10	70	31	73	46	74
		30	70	38	71	43	85
2	8.2	2	70	26	55	36	67
		10	70	29	53	31	83
3	5.4 → 8.2	2	70	23	46	(46)[a]	85
		10	70	23	45	(48)[a]	95
4	None	1 hr	5	25	35	(3)[a]	50
		19 hr	5	27	(15)[a]	(23)[a]	60

[a] These figures represent gains in milk constituents.

milk solids not fat. The usefulness as a fallout countermeasure is limited to the removal of radiostrontium (Sr-89 and Sr-90). The utilization of milk, if treated with calcium phosphate, would likely be limited to nonbeverage products, possibly as dried milk. Since marked deficiencies resulted in the protein (in most cases, also in Ca) of the treated milk, successful manufacture of sterilized milks and of cheese would be questionable.

Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

REFERENCES

(1) Edmondson, L. F. 1964. Ion Exchange Processes for Removing Radioactive Contamination from Milk. J. Dairy Sci., 47: 1201.
(2) Silverman, J. 1960. ACS Meeting, Washington-Maryland Section, spring.